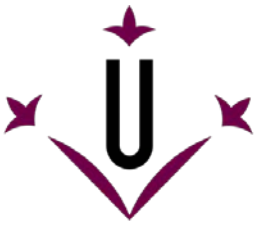

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1 **The stable isotope ecology of terrestrial plant succession**

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Abstract

We review the relevance and use of stable isotopes for the study of plant community succession. Stable isotope measurements provide information on the origin of resources acquired by plants, the processes governing resource uptake and transformation, and the physiological and environmental conditions of plant growth. When combined with measurements of the stable isotope ratio values of soil microbial biomass, soil organic matter and plant litter, isotope measurements of plants can indicate effects of successional changes on ecosystem processes. However, their application to problems of plant succession and ecosystem change is limited by the degree to which the underlying assumptions are met in each study, and complementary measures may be required depending upon the question of interest. At first we discuss the changes that occur in the stable isotope composition of plants and ecosystems with ontogeny and species replacements, as well as their potential evolutionary significance. Secondly, we discuss the imprints of plant competition and facilitation on leaf and wood tissue, as well as how stable isotopes can provide novel insights on the mechanisms underlying plant interactions. Finally, we discuss the capacity for stable isotope measurements to serve as a proxy record for past disturbances such as fire, logging and cyclones.

Keywords: carbon isotopes; disturbances; hydrogen isotopes; nitrogen isotopes; ontogeny; oxygen isotopes; plant-plant interactions; species replacements.

Introduction

Isotopes are variants of an element with identical number of protons in their atoms, but differing in the number of neutrons and atomic masses. Unlike radioisotopes, the nuclei of stable isotopes do not undergo radioactive decay. For a given element, the light isotope (with smaller numbers of neutrons) is often at least two orders of magnitude more abundant than its heavy counterpart(s). For most elements of biological interest (e.g., H, C, N, O, S), the abundance of the light isotope relative to that of the heavy isotope is altered by biotic and abiotic processes. Therefore isotope ratio measurements of biological compounds indicate origin and process information (Williams et al. 2007, Resco et al. 2010). Indeed, the ecological applications of stable isotopes have exponentially grown within the last few years (Figure 1a). Examination of the latest 280 articles with the expression 'stable isotop* eco*' in Web of Science showed that the application of stable isotopes for the study of community and population dynamics is relatively common (Figure 1b). However, no study has yet, to the best of our knowledge, evaluated in a synthetic manner applications of stable isotope measurements in studies of plant succession in terrestrial ecosystems.

Succession, the process whereby species populations colonise, grow and become extinct at a given site has long been a central, organising theme within plant community ecology (Clements 1936; Gleason 1926; Bazzaz 1979; Cutler 2010). The process of plant succession is nearly universal and is largely driven by ontogenetic and growth changes, biotic interactions and disturbances. Because succession within different ecosystems is governed by different processes, our discussion is largely focused on temperate and warm xeric environments, although the main caveats and conclusions are likely to apply to other ecosystems too.

Here we review the stable isotope ecology of plant succession. That is, we

explore the imprint of succession and associated processes on the stable isotope composition of ecosystems, and how stable isotope signatures may help us better understand this process. Our goal is not to discuss the different alternative views currently existing on succession, as these have been dealt with in other studies (Cutler et al. 2008). Instead, our review begins with an explanation and discussion of stable isotope nomenclature and theory. We then move on to discuss the information provided by stable isotopes on physiological and biogeochemical changes that occur during plant ontogeny and growth and species replacements. In the next section, we discuss stable isotopes as tracers and indicators of plant interactions. Finally, we synthesize the role of isotopes to identify disturbances and short-term post-disturbance processes.

Stable isotope theory

The different isotopes of an element are denoted by a superscript preceding its chemical symbol that indicates the atomic mass number, which is approximately the sum of the number of protons and neutrons (i.e. ^{1}H or ^{2}H). The isotope composition of a sample for light elements is typically expressed as a ratio relative to an internationally recognised standard material using the δ notation:

$$\delta X\ (‰) = (R_{sample}/R_{standard} - 1) * 10^{3} \qquad (1)$$

where X represents the isotope of interest and R the ratio of the heavy to the light isotope.

It is sometimes convenient to express the isotope composition in terms of discrimination (Δ), which reflects the magnitude of isotopic partitioning between reactant or source, and product. Depending upon the question of interest, either

$$\Delta = \left(\frac{\delta_{source} - \delta_{sample}}{1 + \delta_{sample}/10^{3}} \right) 10^{3} \qquad (2)$$

or

$$\Delta = \delta_{source} - \delta_{sample} \quad (3)$$

have been used. As these equations are not mathematically identical, they may sometimes yield different results. It is thus important that authors specify which equation was used to calculate Δ.

$\delta^{13}C$ and $\delta^{18}O$ in bulk leaf tissue

Carbon isotope composition is perhaps the most widely measured isotopic signature of plant communities. The carbon isotope discrimination that occurs during photosynthetic gas exchange in C_3 plants was described by (Farquhar et al. 1982, Farquhar and Richards 1984, Farquhar et al. 1989):

$$\Delta = a_b \frac{p_a - p_s}{p_a} + a \frac{p_s - p_i}{p_a} + (b_s + a_w) \frac{p_i - p_c}{p_a} + b \frac{p_c}{p_a} - \frac{\frac{eR_d}{k} + f\Gamma^*}{p_a} \quad (4)$$

where the fractionation factors are a_b, a, a_w, b_s and b for CO_2 diffusion through the boundary layer (2.9‰), stomata (4.4‰), water (0.7‰), CO_2 entering solution (1.1‰) and the net fractionation of phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase activity (estimated at 27‰; Roeske and O'Leary 1984), respectively. Carbon dioxide partial pressures (Pa) are denoted by p_a, p_s, p_i and p_c at the atmosphere surrounding the leaf, at the leaf surface, in the intercellular spaces and at the sites of carboxylation, respectively. The variables Γ^*,R_d, k, f and e represent the CO_2 compensation point (Pa) in the absence of day respiration, day respiration rate (μmol m^{-2} s^{-1}), carboxylation efficiency (μmol m^{-2} s^{-1} Pa^{-1}), and fractionations associated with photorespiration and day respiration (‰) respectively. The first three terms of eq. 4 indicate fractionations during the diffusion of CO_2 from the leaf surface

to the carboxylation site, the fourth term indicates fractionations during carboxylation, and the last term the effects of respiration.

This model has often been simplified based on the correlation that is generally observed between Δ and p_i/p_a:

$$\Delta = a + (b - a)\frac{p_i}{p_a} \quad (5)$$

Where this simplification holds, C_3 photosynthetic carbon isotope discrimination varies linearly with p_i/p_a and can be used as a proxy for water-use efficiency (WUE), the ratio of carbon assimilation (A) to transpiration (E), provided that leaf to air vapour pressure deficit (D) remains constant:

$$\frac{A}{E} = \frac{p_a\left(1 - \frac{p_i}{p_a}\right)}{1.6D} \quad (6)$$

This carbon isotope discrimination model for C_3 plants is a steady-state gas exchange model. However, it has been widely applied to the analysis of leaf bulk tissue as a proxy of WUE. Lack of temporal stability in D under natural conditions and for different species (Smith 1978), differences in mesophyll conductance to CO_2 and in the importance of fractionation processes in respiration or post-photosynthetic processes may introduce error when using Δ as a proxy of WUE, unless additional measurements are performed (Salmon et al. 2011, Cernusak et al. 2009, Bickford et al. 2009, Seibt et al. 2008, Warren and Adams 2006). In this review, our analysis of $\delta^{13}C$ as a proxy for WUE is largely based on studies that validated the potential correlation between $\delta^{13}C$ and WUE.

To disentangle whether changes in WUE are due to changes in A or in E, $\Delta^{18}O$ (defined as enrichment above source water, eq 2) and leaf N concentration are often used. Whereas $\Delta^{18}O$ is affected by stomatal conductance (g_s) but not by A, leaf N is

often correlated with A and independent from g_s. The use of $\Delta^{18}O$ as a proxy of stomatal behaviour in multi-species comparisons has been called into question recently if changes in leaf hydraulic properties affecting the path of transpiration flow (L) are not considered. L indicates the water pathway to the evaporation site and increases with decreasing water availability, likely because of reduced mesophyll hydraulic conductance, which leads to reduced $\Delta^{18}O$ under water scarcity (Ferrio et al. 2009; Kahmen et al. 2008). Since changes in L are species-specific, lack of understanding variation in L across species could compromise the validity of using $\delta^{18}O$ to infer changes in g_s across multi-species comparisons (Ferrio et al. 2009).

$\delta^{18}O$ and δD in xylem sap

Another widespread application of stable isotopes for the study of plant succession is their use in disentangling the source of water used by plants (Ehleringer and Dawson 1992, Ogle and Reynolds 2004, Williams and Ehleringer 2000). Within a soil profile, a distinct composition of δ^2H and $\delta^{18}O$ at different depths is often observed, mainly because: 1) evaporative enrichment of heavy isotopes occurs at surface soil layers; and 2) rains falling during the colder season have a different isotopic composition from rain falling in warmer seasons because of Rayleigh distillations (Clark and Fritz 1997) and, whereas the former will tend to percolate deep within the soil profile, a large proportion of the latter will be evaporated briefly after water inputs. The isotope composition of xylem sap corresponds to the isotope composition of the source in the absence of fractionation during uptake (Dawson and Ehleringer 1993). Thus, it has been a common practice to compare the isotope composition of xylem sap across species to determine the potential overlap in water sources in coexisting species (Ehleringer et al. 1991).

The assumption of no fractionation during water uptake at the soil-root interface in halophytic and xerophytic plants has been questioned (Ellsworth and Williams 2007, Lin and Sternberg 1993). Significant (3‰ to 9‰) isotopic separation ($\Delta^2H = \Delta^2H_{soil\ water} - \Delta^2H_{xylem\ water}$) was observed in 12 halophytic and xerophytic plants from the southwestern USA (Ellsworth and Williams 2007). However, further studies are necessary to clarify the widespread occurrence of fractionation during water uptake, the magnitude of the isotopic separation and the underlying mechanism. For instance, these results could be associated with problems during cryogenic vacuum distillation, the most common method used to extract water from stem tissues (Ehleringer et al. 2000). Moreover, apparent fractionation during water uptake is expected to disappear over longer time frames, as steady-state is approached, which was not observed in the short-term observations by Ellsworth and Williams (2007). Meanwhile, it is advisable for studies in xeric and saline environments to at least perform a sensitivity analysis or estimate the uncertainty associated with the assumption of no fractionation during water uptake.

$\delta^{15}N$ in bulk leaf tissue and soils

Changes in species composition across succession lead to nutrient redistribution (Walker et al. 2010, Wardle et al. 2004). In the absence of abrupt phase transitions or catastrophic disturbances, nutrient limitations often develop, which may be followed by determining $\delta^{15}N$. The pattern of change across ecosystems in internal flows and output losses of N have been approached either by measuring ^{15}N natural abundance or by artificially introducing N with a known (usually highly enriched) isotope composition (labelling; Robinson 2001). Natural abundance measurements require smaller economic input. However, current natural abundance if ^{15}N results from the long-term ecological

processes with an indefinite amount of fractionation processes which, in turn, greatly influence stable isotope composition. Therefore, this approach only allows for a relative assessment of differences in the 'leaky-unleaky' character (that is, openness) of the N cycle among ecosystems. Enrichment methods are not affected by these longer-term effects. Biological or physical fractionations have a limited effect on labelled samples because the isotope composition of labelled samples is one or more orders of magnitude higher than the magnitude of fractionations (Mary et al. 1998). Thus, labelling allows for tracking specific N flows in present conditions, increases the detection sensitivity of the process of interest, and minimises the importance of errors associated with fractionation estimations.

The stable isotope signature of plant succession

Self-organisation across successional change often leads to a slowing down in turnover rates of chemical elements and an increase of their control by the organisms (Margalef 1997). Higher turnover of lighter bonds leads to preferential breakdown and excretion of lighter elements, while the relative concentration of the heavy mass isotope in the local stores of materials synthesised by life processes increases (Figure. 2). At first, changes in the isotopic composition of plants with age are discussed, followed by changes in the isotopic signature of species composition in the absence of disturbance and finally the insights provided by stable isotopes into biogeochemical changes occurring throughout succession are drawn together.

Ontogenetic changes

As succession advances in the absence of disturbances, the demographic age structure shifts towards an increasing proportion of older individuals. The effects of

ageing on plant physiology have been amply reviewed (Bond 2000, Gereenwood 1995, Munné-Bosch 2008, Ryan et al. 2006, Zimmerman 1972), but its interplay with population and community dynamics has been less explored due to its inherent complexity. Here, we focus on how the composition of stable isotopes can be used to estimate changes in the developmental stage of the plants and to infer whether the changes in traits involved are adaptive.

Up to four main developmental phases can be observed in plants: 1) seedling phase, after germination; 2) juvenile or sapling phase, before individuals have reached sexual maturity; 3) mature phase, on sexual reproduction; and 4) senescence, with marked visual and growth differences from mature plants; with phases 3 and 4 being merged in clonal plants with vegetative propagation (Bond 2000, Cavender-Bares and Bazzaz 2000). As a common, nearly universal increase in $\delta^{13}C$ (becoming less negative) has been observed with age in plants across a broad variety of ecosystems, $\delta^{13}C$ could be considered as a proxy for developmental phase changes because (Figs. 2, 3; Barnard and Ryan 2003, Cavender-Bares and Bazzaz 2000, Cordell et al. 1998, Delzon et al. 2004, Donovan and Ehleringer 1991, Grulke and Retzlaff 2001, Hansen 1996, Mcdowell et al. 2005, McDowell et al. 2002, Mencuccini et al. 2005, Miller et al. 1995, Sandquist et al. 1993, Sismilich et al. 2003, Yoder et al. 1994, Zotz et al. 2004).

Variation in age is often accompanied by variation in size. Thus, the capacity to access water, nutrients and radiation in younger plants is often more limited than in older plants. This raises the question of whether changes in $\delta^{13}C$ with age could be solely due to differences in size across ages that affect the microclimate experienced by young and older plants alone, or whether physiological and morphological adjustments also occur. In forest ecosystems, for instance, low $\delta^{13}C$ in juveniles could just be reflecting a lower photosynthetic capacity due to shading or access to a limited pool of

nutrients as well as greater use of ^{13}C-depleted carbon dioxide from soil respiration in the surface boundary layer inhabited by juveniles (Cavender-Bares and Bazzaz 2000, Juarez-Lopez et al. 2008). This effect has been factored out using different experimental procedures: 1) with seedlings grown in a greenhouse under conditions that simulate those experienced by adult individuals (Cavender-Bares and Bazzaz 2000, Cordell et al. 1998); and 2) by comparing the short-term changes in $\delta^{13}C$ in phase transitions (Sismilich et al. 2003). These studies have established that a lower $\delta^{13}C$ in seedlings is not solely an artefact arising from differences in resource availability.

A second confounding factor with regard to the covariation between size and age is that the lower $\delta^{13}C$ in younger individuals could be due to hydraulic or other constraints imposed by height (Ryan et al. 2006). However, $\delta^{13}C$ in low stature shrubs, where height-induced hydraulic constraints are very minor, also increases with age (Donovan and Ehleringer 1991, Sandquist et al. 1993). Finally, comparisons across ages could be confounded by differences in the genetic origin of greenhouse grown seedlings and mature field plants, if seeds did not derive from the same site. However, genetic differences due to provenance show a small impact on the different $\delta^{13}C$ values across ages (Cavender-Bares and Bazzaz 2000). These results indicate that increased $\delta^{13}C$ with age is not solely due to the covariation between age and size, but also because leaf morphological and physiological adjustments occur with age.

Differences in $\delta^{13}C$ reflect a changing balance between photosynthetic capacity and CO_2 conductance from the leaf surface to the carboxylation site (p_c/p_a) during gas exchange, and may be correlated to WUE only under constant D. That is, $\delta^{13}C$ provides an integration of the p_c/p_a set point reflecting evolution of potentially a large suite of traits ultimately impacting leaf gas exchange (Farquhar et al. 1989). Since $\delta^{13}C$ has been found lower in seedlings than in adult plants from contrasting phylogenetic and

geographic origins even when they were exposed to the comparable radiation, temperature and nutrition environments (Cavender-Bares and Bazzaz 2000, Cordell et al. 1998), we can discard these environmental factors as the sole drivers of the nearly universal increase in $\delta^{13}C$ with age.

In turn, increasing $\delta^{13}C$ with age may be adaptive if seedlings maximise carbon assimilation (that is, if conductance to CO_2 is very high to assimilate as much carbon as possible, which would lead to increased p_c/p_a and thus lower $\delta^{13}C$ in seedlings) and this leads to increased growth, and if older plants benefit from a more conservative use of resources. There are several possible mechanisms explaining this process. For instance, increasing leaf life-span leads to lower $\delta^{13}C$ (Werner and Máguas 2010). Since short leaf life-span is usually necessary to maximise carbon assimilation (Wright et al. 2004), low $\delta^{13}C$ in seedlings could be adaptive when indicative of a decrease in leaf life-span leading to increased growth. On the other hand, older plants could increase their leaf life-span, at the expense of not maximising carbon assimilation, in a more conservative use of resources. Another possible mechanism was put forward by Donovan and Ehleringer (1991), who hypothesised that high $\delta^{13}C$ in older plants could also be adaptive, when this leads to a decreased risk of drought-induced damage resulting from a high conductance to water vapour.

Changes in species composition

Changes in species composition during succession may be gradual, where initial dominance by pioneer species gradually shifts towards dominance by late successional species ('initial floristics' model, sensu Oliver and Larson [1996]), or they may be abrupt, when a certain tipping point or ecological threshold is reached, leading to a radically different plant community ('relay floristics' model). When species replacement

is gradual, changes in the stable isotope composition of plant communities along successional gradients mirror changes in ontogeny: $\delta^{13}C$ and $\delta^{18}O$ increase from early- to late- successional species, at least in the absence of a shift in the photosynthetic pathway of the plants forming the community (Navas et al. 2010, Wang 2003). Thus, low $\delta^{13}C$ in early succession could also be adaptive if it led to higher growth to enhance establishment, and high $\delta^{13}C$ later in succession could indicate that these species applied a more conservative resource (i.e. water) use strategy. These changes in growth rates and allocation, along with the strategy to withstand water stress have been corroborated in different studies (Bazzaz 1979, Navas et al. 2010).

Stable isotopes have been used to identify tipping points after which a new stable state occurs. Analyses of the carbon isotope composition in palaeological remains of sediments or charcoal, amongst others, have provided insights into the mechanisms responsible for abrupt phase shift transitions in vegetation. These changes are relatively easy to detect when shifts involve changes in photosynthetic pathways, such as the replacement of C_4 grasslands by C_3 (woody) plants in savannas. The photosynthetic apparatus in C_4 species usually results in much more enriched plant material (-10 to -16‰), with a very distinct isotope composition from that of C_3 species (-20 to -35‰) (Farquhar et al. 1989; Sage et al. 2007). For instance, abrupt vegetation changes, from grassland to savannahs have been detected by measuring the organic $\delta^{13}C$ in sediments in the Kruger National Park, South Africa (Gillson and Ekblom 2009). By combining these data with the $\delta^{18}O$ and $\delta^{13}C$ isotopic sequence in a speolothem (secondary mineral deposits in a cave) nearby, the authors were able to explore the mechanisms underlying such phase transition and study resilience shown by the different ecosystems. Whereas savannahs at some of the studied sites showed high resilience to fire and abiotic stressors, savannah to grassland transitions were observed at other sites in response to

changes in the rainfall regime.

Biogeochemical changes

The Nutrient Retention Hypothesis (Vitousek and Reiners 1975) states that nutrient losses from terrestrial watersheds are largely driven by biomass dynamics after disturbance and during succession, especially in forest ecosystems. According to this hypothesis, N loss rates are highest in very early succession (e.g. recent clear fells) and in steady state, late-successional ecosystems, but are negligible in aggrading, maturing forests. This pattern of N loss has been shown to lead increases with time in $\delta^{15}N$ measured in leaves or soils increasing in various ecosystems (Billings and Richter 2006, Brenner et al. 2001, Compton et al. 2007, Hobbie et al. 1998, Hyodo and Wardle 2009, Vitousek et al. 1989). Austin and Vitousek (1998), for instance, reported low $\delta^{15}N$ (1-4‰) in early stages of a Hawaiian primary succession chronosequence, which was interpreted as resulting from small losses relative to atmospheric inputs by biological fixation and dry or wet deposition. As net primary production peaks and declines thereafter during succession, soil $\delta^{15}N$ increases mainly due to the fractionation during N mineralisation and to the increase of N losses in, predominantly, ^{15}N-depleted (N_2O, NO_3^-) material (Handley et al. 1999). In later stages, a depletion in foliar ^{15}N relative to bulk soil has been related to the prevalence of mycorrhizal fractionations, rather than mineralisation (Hobbie et al. 1999).

Changes in ^{15}N natural abundance may also be viewed as a long-term integrator of variability in the openness of the N cycle due to causes other than successional change, such as differences in state factors or land-use history (Eshetu and Högberg

2000). Thus, the potentially confounding effects should be carefully ruled out when considering chronosequences that use spatial variability as a surrogate of time.

In general, $\delta^{15}N$ also increases with depth in the soil profile (by ca. 5 to 10 ‰; Fry 2006). This is likely due to the (1) incorporation of isotopically heavy fungal residues at depth; (2) preferential conservation of ^{15}N enriched compounds during decomposition; and (3) losses of depleted N by denitrification or NO_3^- leaching (Hobbie and Ouimette 2009). However, Makarov et al. (2006) have recently reported the pattern of increasing heavy isotope ratio in total N with soil depth does not apply to NH_4^+-N, at least in alpine and tundra ecosystems. As a possible explanation, higher nitrification and biological immobilisation of 'lighter' NH_4^+-N near the soil surface was suggested; but the exact mechanism responsible for NH_4^+-^{15}N depletion with soil depth is not fully understood yet. However, this finding provides a new approach to disentangle shifts along with succession in rooting-depth niche partitioning among plant species, in terms of NH_4^+-N acquisition, on the base of variability in foliar $\delta^{15}N$ signal.

In addition to measuring natural abundance, ecosystem labelling by artificially introducing ^{15}N-enriched N is useful to directly assess biogeochemical shifts in N input/output budgets occurring during plant succession. The natural abundance of ^{15}N could be heavily influenced by long-term processes which mask the question of interest, a problem that may be overcome by the use of enrichment methods. However, incomplete and even poor recovery is a common feature of many ecosystem ^{15}N tracer studies (Gundersen et al. 1998, Nadelhoffer et al. 2004), which constrains chances of accurately closing the mass balance. Such constraints are often explained by unmeasured or partially measured fluxes such as denitrification and leaching from the soil, and by direct volatilisation and photolysis of nitrogen compounds. There are also

large uncertainties associated with small enrichments of ecosystems pools with high N content, such as the forest floor, soils and bark (Nadelhoffer et al. 2004).

Another kind of biogeochemical shift taking place during succession concerns the nature and the degree of nutritional limitations. Nitrogen limitation is commonly prevalent over much of the successional process, although a decrease in its strength, and eventually a transition toward P limitation, may occur as succession advances (Vitousek and Howarth 1991). Isotopes are also valuable methodological tools in this regard. For instance, a bioassay has been developed to measure isotope uptake rates by field-collected, excised roots under standard laboratory conditions. Uptake rates show a negative exponential relationship with the degree by which plant nutrient demand is being satisfied by soil nutrient supply (Jones et al. 1991). The bioassay has been successfully applied, for instance, in the screening of N to other element imbalances of tree nutrition in European forests subjected to atmospheric N deposition (Harrison et al. 1999), and it was key to support the hypothesis that a deficiency-induction by nutrients other than N is a widespread mechanism triggering the threshold shift from a N-limited to a N-saturated status in forests (Carreira et al. 2000).

Stable isotopes and plant interactions

Above- and/or below-ground biomass generally increases as succession advances, following the establishment of new individuals and plant growth. This leads to an increasing intensity and importance of plant interactions, which may lead to species displacements. Stable isotopes can indicate plant interactions, and may be able to provide information on the underlying processes.

Plant interactions elicit a complex set of responses which may range from short-term physiological responses, to longer-term acclimatory or even evolutionary

adjustments. Resource interactions, an analogy to resource competition (Lambers et al. 1998), but that includes competitive as well as facilitative interactions driven by sharing a common pool of growth-limiting resources, are commonly induced by changes in light, water or nutrient availability resulting from interactions with neighbouring individuals. Stable isotopes have been largely used to elucidate the physiological response to resource interactions driven by changes in water availability. Since water is prevalent in limiting plant productivity worldwide, this section focuses on competition for water.

Studies on the isotopic composition of soil water and xylem sap have provided evidence for either a lack of interactions, competitive interactions, or facilitative interactions, depending on the environment and the species concerned. A comparison of the water extraction depth across different functional types in the semi-desert species of southern Utah (Ehleringer et al. 1991) indicated a spatial separation in the water source between deep-rooted perennials, which largely depended on deep soil water recharged by winter rains, and a succulent CAM plant, which used only water from summer rains. Annual and herbaceous perennial species in the same community were both using soil water from summer rains, and in addition, herbaceous perennial species, partly dependant also on spring and winter recharged water in deeper soil horizons, were also competing for the same water sources as woody perennials.

Hydraulic redistribution of water within the soil profile, where water is passively translocated from higher (e.g., deeper soil) to lower water concentration sites (e.g.: shallow soil), has been documented in a variety of ecosystems and species (Dawson 1993, Hultine et al. 2004, Mooney et al. 1980, Peñuelas and Filella 2003). Differences in the isotopic composition in water with depth of both, δD and $\delta^{18}O$ (see section $\delta^{18}O$

and δD in xylem sap above) provide the basis for using stable isotopes to identify and quantify the amount of hydraulically lifted water.

An early study on the ecological relevance of hydraulic redistribution in a mesic forested ecosystem (Dawson 1993) documented a facilitative effect of hydraulic redistribution. Deep water that was hydraulically redistributed by sugar maple (*Acer saccharum*) was used by neighbouring shallow rooted plants (from 3 to 60% of the total water used by neighbours). Hydraulic redistribution could also drive competitive interactions. For instance, velvet mesquite (*Prosopis velutina*), a phreatophyte with deep tap roots from the American southwest, redistributes soil water that originates from dormant season precipitation from shallow to deep soil layers during the dormant season. This leads to increased water availability for this species during the dry summer, while diminishing the amount of water available during that season for other species with shallower root systems (Scott et al. 2008). More recently, hydraulic redistribution has also been documented as a mechanism of interference competition. In an arid coastal system, *Pistacia lentiscus* was reported to hydraulically redistribute salty groundwater which was later taken up by neighbouring *Juniperus phoenicia*, a species with reported low tolerance to salinity. Photosynthetic gas exchange and survival, for instance, of neighbouring *Juniperus* individuals that were using the salty water hydraulically redistributed by *Pistacia*, was poorer than that of isolated *Juniperus* individuals, with no access to hydraulically redistributed groundwater (Armas et al. 2010). Altogether, these results indicate that, while in mesic environments hydraulic redistribution may facilitate water acquisition of individuals neighbouring the hydraulic 'redistributor' (Dawson 1993), in semi-arid and coastal ecosystems, hydraulic redistribution may serve as mechanism for resource or interference competition (Armas et al. 2010, Scott et al. 2008).

The impact of plant interactions on photosynthesis has been assessed by measuring the C and O isotope composition of leaves (Grams et al. 2007, Ramírez et al. 2009; Table 1). Run-off interception by upslope neighbours led to an increase in intra-specific competition amongst *Stipa tenacissima* individuals in a semi-arid tussock grassland (Ramírez et al. 2009). This was recorded in the isotope composition of the leaves, where an enrichment in foliar $\delta^{18}O$ and $\delta^{13}C$ was observed as the degree of canopy overlap across neighbour individuals increased (Fig. 4), which correlated with decreases in both stomatal conductance and assimilation as competition intensity increased. However, Powers et al. (2010) observed an increase $\delta^{13}C$ after competition removal by harvest in *Pinus resinosa* but no change in $\delta^{18}O$, reflecting an increase in photosynthesis but no effect on g_s. Others have found that competition did not impact either $\delta^{13}C$ or $\delta^{18}O$ (Table 1).

The effects of competition on photosynthetic gas exchange across the whole life of a plant may be assessed by examining the isotope composition in tree rings. Linares et al. (2009) observed how $\delta^{13}C$ became progressively more enriched with age in *Abies pinsapso*, when increased plant growth during succession lead to higher competition intensity. Values of $\delta^{13}C$ can help identify the resource that plants are competing for (e.g. water vs light): whereas shading usually leads to ^{13}C depletion, water scarcity generally increases the $\delta^{13}C$ enrichment (Grams et al. 2007).

Stable isotopes and vegetation disturbance

Presence and severity of disturbance

Disturbance is, arguably, a major driver of succession. The time, kind, intensity and magnitude of the disturbance exert large influence on the post-disturbance plant community (Bazzaz 1979). As stable isotopes can indicate biological responses to

changes in environmental conditions, for example when matter accretion occurs in a cumulative way such as in tree rings, stable isotopes can provide significant insight on the time, kind, intensity and magnitude of the disturbance.

Amongst the varied types of disturbances that impact ecosystems, an important discussion aims at clarifying the potential link between global change and an increase in frequency of extreme events, such as the occurrence of tropical cyclones and hurricanes (Mora et al. 2007). Increases in the frequency and severity of wildfires and of large-scale vegetation mortality in response to global warming are also expected under global change, although with a smaller degree of uncertainty than increases in tropical cyclones (Alcamo et al. 2007, Breshears et al. 2009).

The presence and intensity of drought and tropical cyclones may be identified by examining the O isotope composition in tree rings. The former leads to a drastic increase in $\delta^{18}O$ as a result of reducing stomatal conductance (Barbour 2007, Mora et al. 2007). Moreover, the isotope composition of water from tropical cyclones is typically very distinct from 'normal' precipitation (by up to 10‰ lower in tropical systems; Lawrence et al. 2002). A study on the tropical cyclone activity in the south-eastern US identified changes in $\Delta^{18}O$ in late wood that indicated increased cyclone activity at different times of the 19th and 20th century (Miller et al. 2006). Changes in $\Delta^{18}O$ were observed only in late wood because, in that area, tropical cyclones often appear late within in the growing season.

It is yet to be established if stable isotopes can indicate the presence and severity of wildfires. Stable isotopes may become a promising tool to determine post-burn changes in soil organic matter (Czimczik et al. 2002, Fernandez et al. 2004, Saito et al. 2007). For example, $\delta^{13}C$ has been proposed as a simple surrogate to quantify the amount of 'black carbon' (Czimczik et al. 2002). Black carbon is a generic term used to

refer to highly condensed organic-C structures generated as a consequence of the incomplete combustion of organic matter by fire (Rovira et al. 2009). In fire-prone ecosystems, the amount of black carbon in the soil is relevant to assess the C balance of fire-prone ecosystems, but quantification methods are time-consuming and different methods may give differing results (Czimczik 2002, Hammes et al. 2007). In forests, the main source of black carbon is wood, which in turn is mainly composed of lignin and cellulose. Depending on burn temperature, the $\delta^{13}C$ of wood tends to decrease, presumably due to the lower thermal stability of the isotopically enriched cellulose when compared to aromatic lignin groups (Czimczik et al. 2002). According to this trend, the $\delta^{13}C$ of black carbon from the soil would be negatively correlated with fire severity, thus providing a ready surrogate for the assessment of fire effects. Indeed, carbonisation experiments have shown that wood becomes more ^{13}C-depleted with increasing the temperature of charcoal formation (Fig. 5), and that most changes occur during the first 15 minutes of heating (Czimczik et al. 2002, Turney et al. 2006). Thus, $\delta^{13}C$ could potentially be a good surrogate for the severity of fire, but mainly in terms of maximum exposure to heat, rather than of fire duration. Nevertheless, there are several concerns about this methodology. The first is that the original $\delta^{13}C$ of wood is variable, not only among species, but also within species depending on their growing conditions (Ferrio et al. 2005, Saurer et al. 2004, Warren et al. 2001). A priori this can be readily overcome, either by obtaining a suitable reference value from non-burned vegetation in the area of study, or by determining the relationship between $\delta^{13}C$-decrease and carbon content increase during carbonisation (Ferrio et al. 2006). A second constraint is that experimental studies on the effect of burning on wood $\delta^{13}C$, although have given comparable trends, showed important quantitative differences (Fig. 5). The chemical composition of wood might play a role, but the lack of consistent differences between

conifers and hardwood species points to the carbonisation environment as the main factor modulating the effect of burning (see Table 2). Unfortunately, carbonisation conditions in wildfires are strongly variable and difficult to reproduce experimentally, and thus a proper validation of the potential use of stable isotopes to identify black carbon has not yet been performed.

Saito et al. (2007) attempted a similar calibration procedure for $\delta^{13}C$ and $\delta^{15}N$ in bulk organic matter in forests soils. Unlike in wood, experimental carbonisation of bulk soil organic matter did not produce a significant change in $\delta^{13}C$, but a progressive increase in $\delta^{15}N$ with increasing temperature and duration of heating. However, testing field samples of soils before, shortly after and 1 year after wildfire gave inconsistent results: either no effect or an increase in $\delta^{15}N$ after fire, and a significant depletion in $\delta^{13}C$. Probably, under field conditions, organic matter changes such as those reproduced in the experiment might be dampened by N_2-fixation and the incorporation of ^{13}C-depleted black carbon into the soil. Thus, although stable isotopes might help to understand the different processes occurring during and after fire, their application is mainly restricted to experimental assays. In this context, soil-labelling approaches appear as the most powerful tools to characterise the fate of the different C and N pools in the soil after fire (Castro et al. 2006, Fernandez et al. 2004) .

Post-disturbance dynamics

Although wildfires are among classic examples of disturbances forcing succession, the particular mechanisms forcing a change in plant communities vary depending on the severity and frequency of fire events (Cattelino et al. 1979, Connell and Slatyer 1977). A direct application of stable isotopes is their use as integrative records of physiological processes to track post-burn plant responses. For example,

Quercus ilex L. is a common sclerophyll tree of the Mediterranean region which intensively resprouts after fire (Terradas 1999). Combining gas exchange measurements over the growing season with measures of leaf $\delta^{13}C$, Fleck et al. (1996) found that young resprouts appearing after fire or tree-fell showed thicker leaves and higher $\delta^{13}C$ (and thus higher water use efficiency). This was further confirmed by different measures of photosynthetic capacity (chlorophyll, N and protein content, RuBisCO activity), which were all consistently higher in disturbed than in control plots. The authors interpreted these changes as a response to increased availability of water and nutrient per shoot mass, allowing greater photosynthetic rates in response to increased radiance. However, since starch is generally enriched with respect to bulk organic matter (Tcherkez et al. 2004), it might be argued that higher $\delta^{13}C$ in resprouts indicates a bigger proportion of C in leaves derived from stored reserves, rather than an increased water use efficiency. As other resprouters, *Q. ilex* is thought to rely on belowground reserves for resprouting after disturbance (Terradas 1999). Although this is clearly the case for C, the origin of N during initial recovery is less clear: although high N losses occur during fire, increased N mineralisation after burn often result in higher nutrient availability (Vallejo et al. 2004). A ^{15}N-labelling experiment with *Q. ilex* seedlings under different N regimes showed that the initial source for resprouting was N stored in the roots, rather than direct N uptake from the soil (El Omari et al. 2003). The study showed a two-phase pattern in N remobilisation during resprouting: firstly, N from the roots is remobilised to form new shoots, and secondly, root reserves are replenished with N from the soil. Thus, although soil-derived N is not directly used for the initial growth of resprouts, it is necessary afterwards to refill root and leave N reserves. As pointed out by Vallejo et al. (2004), this strategy is beneficial for the plants as long as wildfires occur at intervals long enough to allow the nutrient reserve in the soil to

recover, but might imply a loss in long-term ecosystem productivity if wildfires become frequent.

Together with fire frequency, the recovery of N pools in the soil would depend on the ability of legumes appearing during early successional stages to fix atmospheric N_2 (Casals et al. 2005, Hamilton et al. 1993, Hendrick and Pregitzer 1993). Although N_2 fixation is expected to be stimulated after fire due to high light availability, this effect might be counteracted by higher N availability in the soil inhibiting N_2 fixation (Vitousek and Field 1999). The simplest way to assess the amount of N derived from atmospheric N_2 is to take advantage of natural differences between N_2 and other N sources (natural abundance, Hamilton et al. 1993, but see Handley and Scrimgeour 1997). Following this approach, Hamilton et al. (1993) studied N_2-fixation rates in understory legumes of a eucalypt forest in Australia over a period of 27 months following a prescribed fire. Fixation rates showed a substantial increase (up to 30-fold) in N_2-fixation rates per plant after fire, although the estimated contribution of atmospheric N_2 to the whole ecosystem was low due to low plant densities after fire. The natural abundance method can only be applied if the potential sources of N show contrasting $\delta^{15}N$ values. Alternatively, N_2 fixation can be assessed by adding ^{15}N-labelled compounds to the soil. Casals et al. (2005) applied this method to assess to what extent N_2 fixation was enhanced after fire in a grassland, a shrub-grassland and a shrubland, formed as early succession communities in abandoned agricultural lands in north-east Spain. In all cases, they found that most of N in legume species was derived from atmospheric N_2, with grassland communities having slightly higher N_2 fixation than shrublands. Thus, the contribution of N_2-fixation to soil N after fire is likely to increase in closed canopy communities (from grasslands to shurblands to forests), not

only due to a greater legume biomass, but also due to increased competition for soil N resources, compared with open communities.

Conclusions

The potential of stable isotopes to indicate, integrate, record and trace fundamental ecological processes is of great use for the study of plant succession. Ontogenetic changes can be indicated by $\delta^{13}C$. Averaged across phylogenetically distant plant species we observed that $\delta^{13}C$ increased by 1.13‰ from juvenility to maturity. Further work is required to unveil the potential evolutionary significance, if any, of this ontogenetic change in $\delta^{13}C$. With regard to plant-to-plant interactions, changes in the intensity of competition can be studied by $\delta^{13}C$ leaf or tree ring tissue, with higher competition intensity leading to less negative $\delta^{13}C$. Understanding what resource are plants competing for has been for long a focus of research in stable isotope ecology; however a possible fractionation during water uptake has hindered further progress in this area. The intensity of disturbances such as logging also affect $\delta^{13}C$, but if the severity of fire may be traced back by examining $\delta^{13}C$ in plant or soil remains is not clear and $\delta^{13}C$ does not seem a good surrogate for fire severity. Instead, great insights on the openness of the N cycle at different moments in succession and after fire may be gained from studying $\delta^{15}N$, although poor rates of recovery may seriously compromise these studies.

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Notes on contributors

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940 **Table 1.** Effects of intra- (A) or inter- (B) specific competition may lead to increases
941 (↑), or no (=) changes in $\delta^{13}C$ and $\delta^{18}O$ of leaf bulk tissue. NA indicates that data was
942 not available.

Competition was measured as the effect on:	Species	$\delta^{13}C$	$\delta^{18}O$	Reference
(A) Basal-area increment	*Pinus nigra*	=	=	(Martín-Benito et al. 2010)
(B) Physiological status	*Juniperus phoenicea*	=	NA	(Armas et al. 2010)
(B) Tree diameter growth rate	*Diospyros sandwicensis*	=	NA	(Cordell and Sandquist 2008)
(B) Crown volume per biomass	*Picea abies*	=	=	(Grams et al. 2007)
(A) Predawn water potential	*Pinnus halepensis*	↑	=	(Querejeta et al. 2008)
(B) Grain yield	*Zea mays*	↑	NA	(Pansak et al. 2007)
B) Crown volume per biomass	*Fagus sylvatica*	↑	↑	(Grams and Matyssek 2010)
(A) Green biomass	*Stipa tenacissima*	↑	↑	(Ramírez et al. 2009)
(A) Basal-area increment	*Abies pinsapo*	↑	NA	(Linares et al. 2009)
(A) Radial growth	*Pinus resinosa*	↑	=	(Powers et al. 2010)

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Table 2. Comparison among the different charring treatments reported in the literature to assess the effect of fire on wood $\delta^{13}C$. Data source: 1, Jones and Chaloner 1991; 2, Czimczik et al. 2002; 3, Ferrio et al. 2006; 4, Turney et al. 2006; 5, Ferrio et al. 2007.

Heating method	Sample type (size)	Warm-up	Atmospheric conditions	Temperature (duration)	Source
Muffle	Wood block (60 x 25 x 15 mm)	20-55 min	Anaerobic (sand-buried)	180-600ºC (60 min)	1
Muffle	Ground wood (0.3 g)	no	Anaerobic (Argon)	150-480ºC (900 min)	2
Muffle	Incremental core (Ø 5.5 mm x 10-80 mm)	25-35 min	Anaerobic (sand-buried)	300-500ºC (30 min)	3
Muffle	Ground wood (1-3 g)	no	Anaerobic (alu-foil wrap)	200-800ºC (30-240 min)	4
Heat gun	Ground wood (1-3 g)	no	Aerobic (open air)	350 ºC ('till charring')	4
Muffle	½ incremental core (Ø 12 mm x 10-30 mm) ¼ branch slide (Ø 20-40 mm x 10-20 mm)	25-35 min	Anaerobic (sand-buried)	300-500ºC (30 min)	5

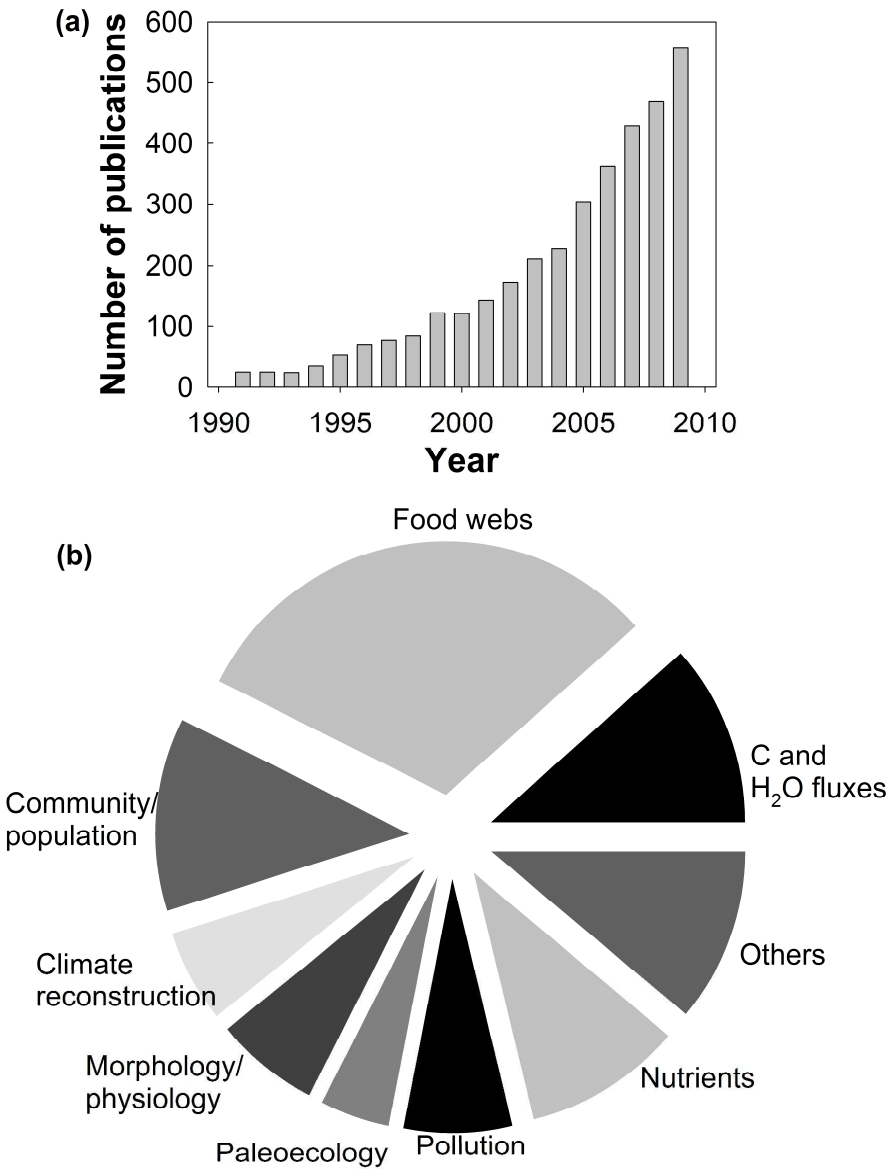
Figure 1. a) Number of publications with the expression 'stable isotope* eco*' as topic in Web of Science (http://www.isiknowledge.com/; April 27th, 2010). **b)** Distribution of the last 280 manuscripts (7.5% of total manuscripts) into categories.

Figure 2. Conceptual figure of changes in the stable isotope composition of ecosystems along succession in ecosystems dominated by C_3 plants. Changes are due to ontogenetic effects and also to species replacements.

Figure 3. Changes in $\delta^{13}C$ (‰) observed in the literature (references in the text) from juvenility to maturity.

Figure 4. Effects of increasing intra-specific competition on the stable isotope composition of bulk leaf in the tussock grass *Stipa tenacissima*. The x-axis reflects the degree of overlap amongst two adjacent individuals. Error bars represent standard errors, and the line is the result of least-squares fitting. Modified from Ramírez et al. 2009.

Figure 5. Effect of charring at different maximum temperatures on wood $\delta^{13}C$ of pines (A), oaks (B) and other hardwood species (C). Experimental charring was generally performed in a muffle furnace under anaerobic conditions. Treatment details are summarized in Table 2. Data source: 1) Jones and Chaloner 1991; 2) Czimczik et al. 2002; 3) Ferrio et al. 2006; 4) Turney et al. 2006; 5) Ferrio et al. 2007.



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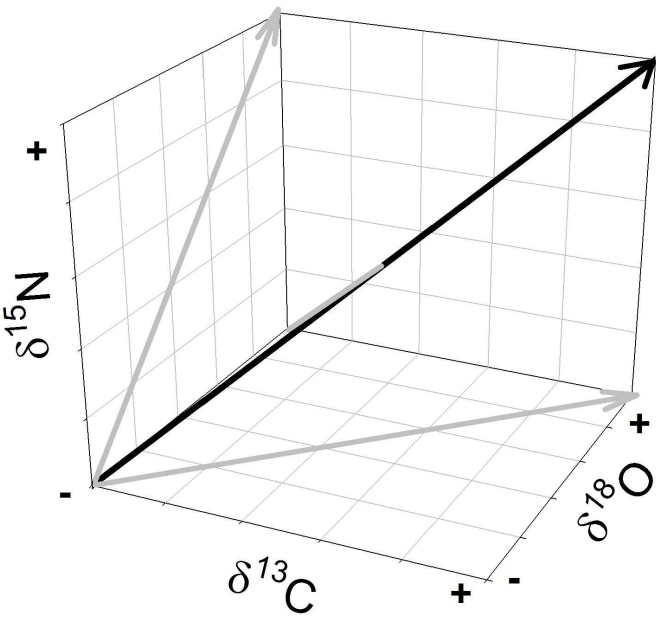

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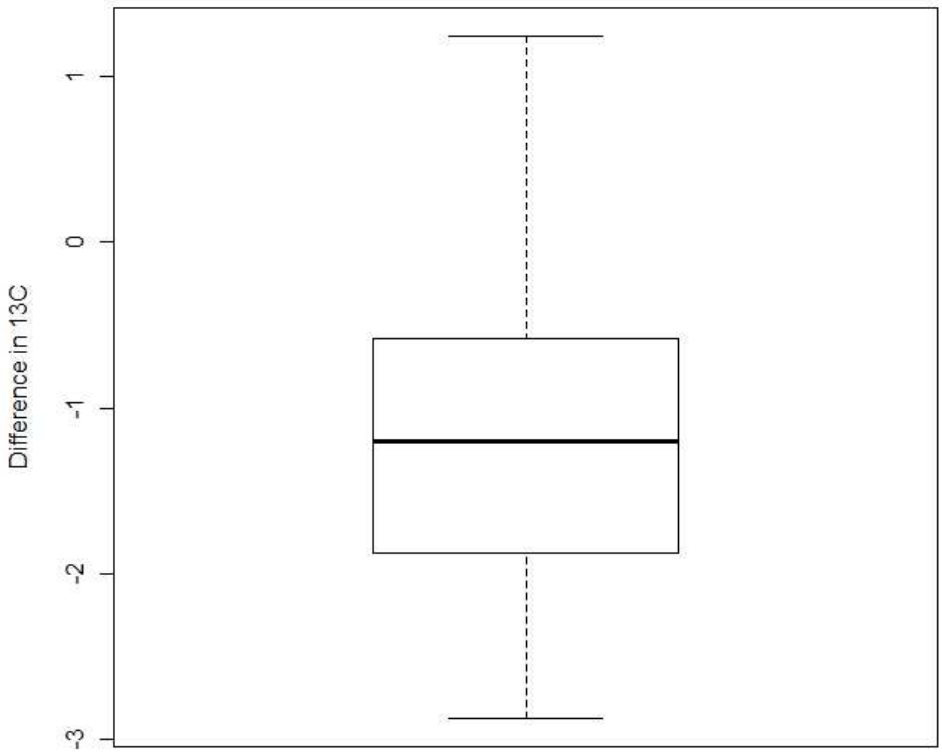

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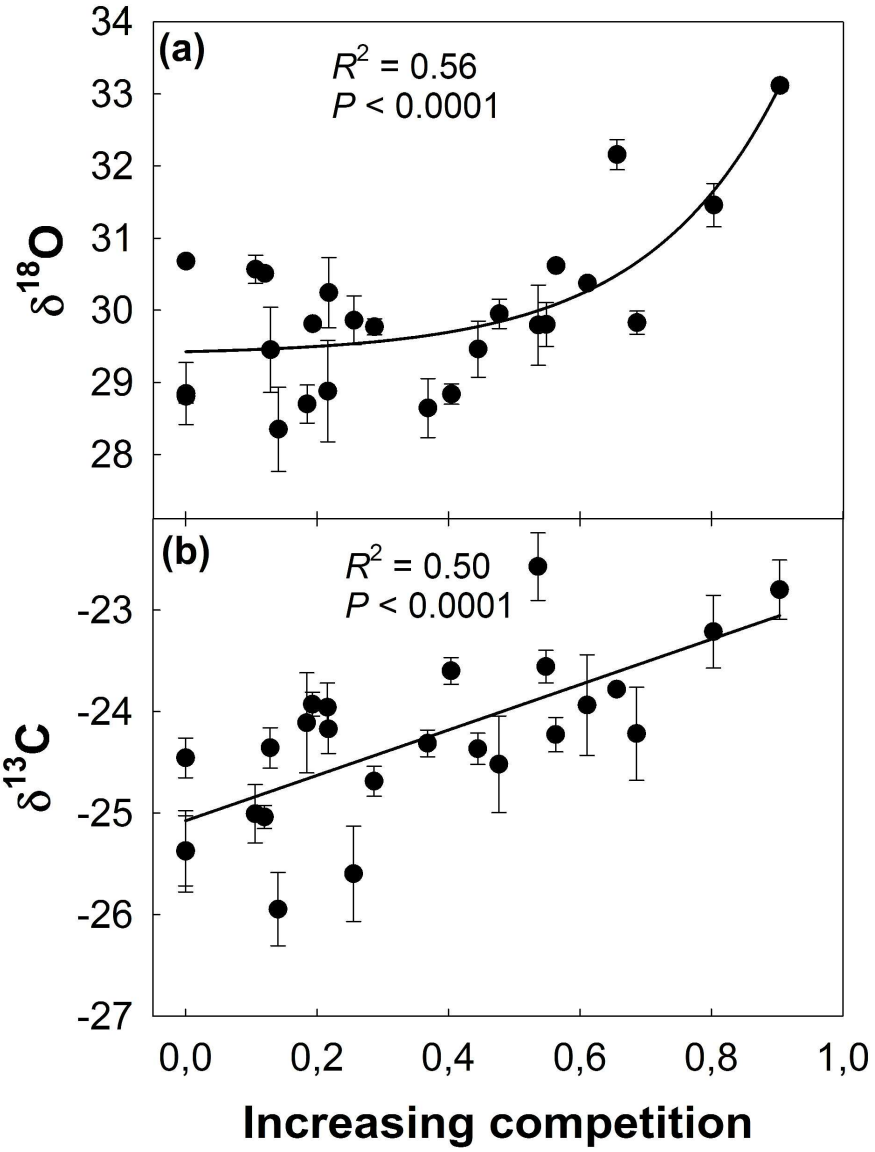

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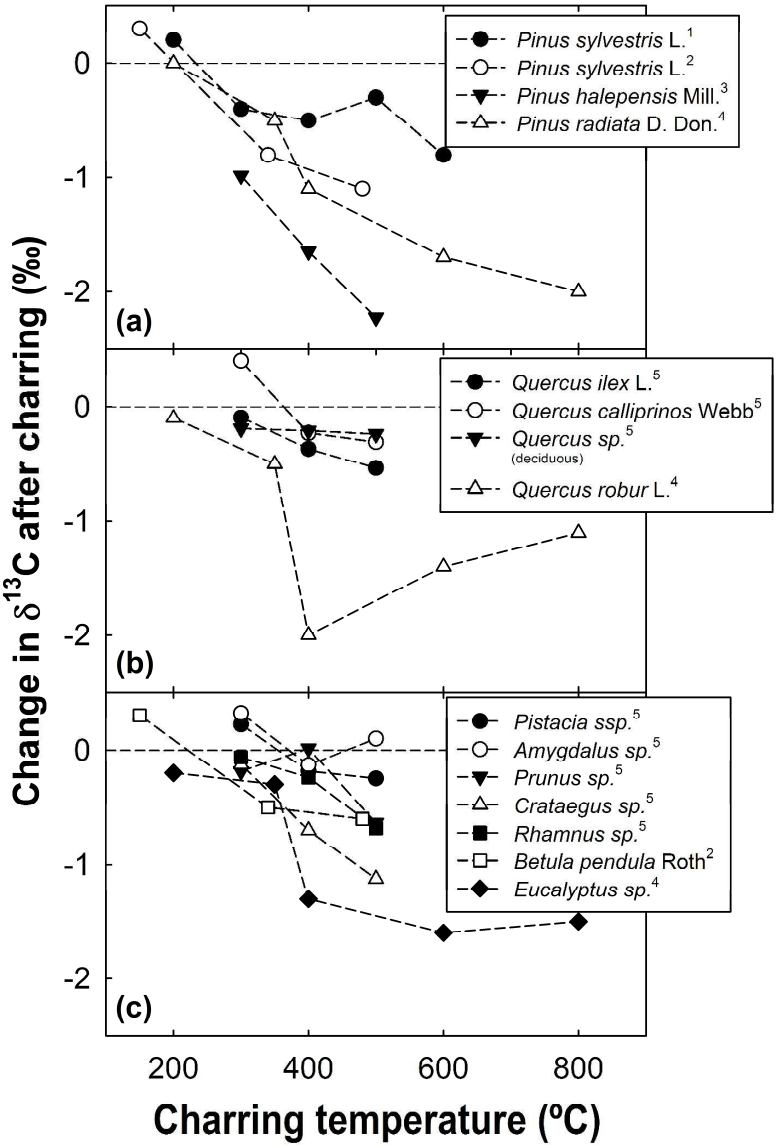

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